CASE REPORT

CFTR Mutation Analysis of a Caucasian Father with Congenital Bilateral Absence of Vas Deferens, a Taiwanese Mother, and Twins Resulting from ICSI Procedure

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Cystic fibrosis (CF), caused by mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene, is one of the most common autosomal recessive diseases in Caucasians. We screened for the *CFTR* gene mutation in a Caucasian father with congenital bilateral absence of the vas deferens (CBAVD), a Taiwanese mother, and twins resulting from an intracytoplasmic single sperm injection (ICSI) procedure. DNA fragments that showed abnormal banding patterns on temporal temperature gradient gel electrophoresis analysis followed by analysis of DNA sequence was used. The Caucasian father with CBAVD had ΔF508 and p.L375F mutations. The two children were heterozygous for the ΔF508 and p.L375F mutations, respectively. Mutation analysis of the *CFTR* gene should always be recommended for infertile couples seeking ICSI. The possibility of the children resulting from ICSI being a victim or carrier of CBAVD or CF, especially when the father is Caucasian with CBAVD, should be discussed during genetic counseling. [*J Formos Med Assoc* 2008;107(9):736–740]

Key Words: *CFTR* mutation, congenital bilateral absence of the vas deferens, cystic fibrosis, intracytoplasmic single sperm injection, vas deferens

Although cystic fibrosis (CF) is one of the most common autosomal recessive diseases in Caucasians, it is very rare in Asian populations.[1,2] Congenital bilateral absence of the vas deferens (CBAVD) is a newly recognized primarily genital phenotype of CF; however, it is relatively common in our practice among male infertility patients with obstructive azoospermia.[3] A recent survey on a small number of Asian patients with CF transmembrane conductance regulator (*CFTR*) gene mutations revealed mostly unique mutations that have never been reported in Caucasian CF patients.[2] Our previous study also showed that none of the major *CFTR* gene mutations such as ΔF508 or p.R117H could be identified in 27 Taiwanese males with CBAVD. A high frequency of 5T alleles (44%) in these patients was also noted in comparison to Caucasian patients with CBAVD.[4,5]

We also reported five novel mutations of the *CFTR* gene found in five of 36 patients with CBAVD using temporal temperature gradient gel

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Received: May 9, 2007
Revised: May 31, 2007
Accepted: August 7, 2007

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electrophoresis (TTGE). The overall frequency of *CFTR* mutant alleles in Taiwanese CBAVD males is lower than that in CBAVD patients of other ethnic origins (36% *vs.* 50–74%). According to these results, we thought that the risk of siblings of CBAVD patients resulting from modern assisted reproductive technologies such as intracytoplasmic single sperm injection (ICSI) being a victim or carrier of CBAVD or even CF might be lower. However, marriages among different ethnic groups are becoming more common in our society, and this may result in more variable possibilities for transmitting *CFTR* mutant genes to the next generation through ICSI techniques. Herein, we report the case of a Caucasian male with CBAVD who married a Taiwanese woman and, through successful ICSI treatment, had twins. The mutation analysis of their *CFTR* genes can serve as a reference for genetic counseling in the future.

Case Report

The man, of Caucasian descent, was 40 years old; his wife, of Taiwanese descent, was 30 years old. They had a clinical history of infertility for 2 years. During physical examination, the man was found to have CBAVD. Family history was unremarkable for any specific pulmonary infection or a history of bronchiolitis organizing pneumonia. The patient had no digestive symptoms related to pancreatic disorder as a symptom of CF. Semen analysis showed complete azoospermia with a low semen volume (< 1 mL) and a low seminal pH of 6.0–6.5. Transrectal ultrasound revealed bilateral hypoplasia of the seminal vesicle. Both kidneys were normal on renosonography. Both testicular size and hormone profile showed that he had normal spermatogenesis. He had been asymptomatic for CF and had never been checked by an internist. A sweat chloride test was performed; the chloride concentration was 51 mmol/L (normal range, < 60 mmol/L). Fertility checks of his wife by a gynecologist found nothing unusual.

During the ICSI cycle, the wife received clomiphene/hMG/GnRH antagonist protocol as has been previously described.[6] When the two leading follicles reached a diameter of 18 mm, hCG was injected, and transvaginal oocyte retrieval was performed 36 hours later. In total, 10 oocytes were retrieved. Percutaneous epididymal sperm aspiration was performed on the husband by means of a butterfly needle connected to a 10-mL syringe containing 2 mL of HEPES-buffered human tubal fluid (HTF) medium (Irvine Scientific, Santa Ana, CA, USA). The epididymal fluid was aspirated into the medium. The procedure was repeated to aspirate as many spermatozoa as possible. The aspirated fluid was observed for the presence of motile spermatozoa under an inverted microscope. The aspirate containing sperm was pooled together. The final concentration of sperm was adjusted to 2–3 motile spermatozoa per high powered field (400×). We directly aspirated 1 μL of the final pellet of the washed spermatozoa into a peripheral droplet containing 10 μL of HEPES-buffered HTF medium with 0.5% human serum albumin (Irvine Scientific) in a 5-cm petri dish (Falcon 1006; Becton Dickinson, Lincoln Park, NJ, USA) covered by paraffin oil (Sigma, St Louis, MO, USA). Seven to eight spermatozoa were picked up from the above droplet with an injection pipette into another central droplet. The spermatozoa were then aspirated into a polyvinylpyrrolidone drop for immobilization. The ICSI procedure was performed as described by Van Steirteghem et al[7] and Palermo et al[8] with more aggressive immobilization of the spermatozoa than usual. Eight of the 10 eggs were in metaphase II and injected. Injected oocytes were cultured in P-1 medium (Irvine Scientific) supplemented with 10% synthetic serum substitute (Irvine Scientific) in an atmosphere of 5% CO_2 in air and examined for the formation of two pronuclei at 16–18 hours after the ICSI procedure. Six of the injected oocytes were fertilized. The fertilized oocytes were cultured for another 24 hours. Three embryos were transferred on day 2. The wife became pregnant, and a twin pregnancy was shown on transvaginal ultrasonography at 7 weeks of gestation. Her pregnancy was smooth, and she delivered two male babies (with body weights of

Table. *CFTR* gene mutations of all members of this family

	CFTR gene mutation		
	Exon 8	Exon 10	IVS8-Tn
Father: Caucasian CBAVD patient	p.L375F	ΔF508	7T/9T
Mother: Taiwanese	None found	None found	7T/7T
Son A	None found	ΔF508	7T/9T
Son B	p.L375F	None found	7T/7T

2445 and 2430 g, respectively) at 36 weeks of gestation. At birth, there was no respiratory distress or meconium ileus in either of the boys. Both of them had normal external genitalia including testes and vas deferens.

Mutation analysis of the *CFTR* gene was done by the TTGE technique for unknown mutations in the exons and intron–exon junctions of the entire *CFTR* gene.[2,9,10] The primer sequences used for the amplification of the 27 coding exons and their flanking intron–exon junctions, as well as PCR and TTGE conditions have previously been described in detail.[9] Briefly, 5 mL of denatured and reannealed PCR products were loaded onto a polyacrylamide gel containing 6 mol/L urea. Electrophoresis was carried out at 130 V at constant temperature increments of ~1–2 °C/hr over a range of temperatures suitable for each exon.[9] The temperature range of TTGE for each PCR fragment was determined empirically with the aid of a computer simulation (MacMelt, Bio-Rad Laboratories, Hercules, CA, USA).[9,10] The gels were stained in 2 mg/mL ethidium bromide for 5 minutes and imaged with a digital charged-coupled device gel documentation system. TTGE analysis revealed homozygous changes as a band shift and heterozygous changes as multiple bands.[9,10] The DNA fragments that showed abnormal banding patterns on TTGE analysis were sequenced using the Big Dye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA) and analyzed on an ABI Prism 377 DNA Sequencer (Applied Biosystems) according to the manufacturer's protocols. The sequencing data were analyzed using ABI DNA sequencing analysis software

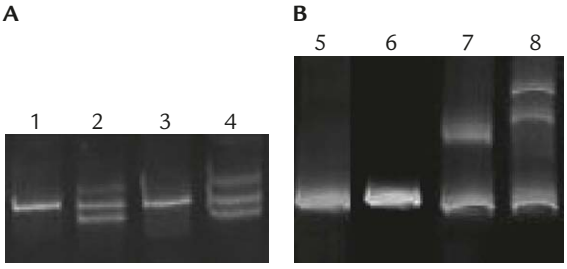

Figure 1. (A) Detection of exon 8 by TTGE: lane 1 = wild type control; lane 2 = son B, heterozygous mutation; lane 3 = son A, wild type; lane 4 = Caucasian father, heterozygous mutation. (B) Detection of exon 10 by TTGE: lane 5 = wild type control; lane 6 = son B, wild type; lane 7 = son A, heterozygous mutation; lane 8 = Caucasian father, heterozygous mutation.

(version 3.0) and compared with GenBank sequences using Mac Vectore (version 7.0). The mRNA (GenBank NM_000492.2) sequence of the *CFTR* gene was used as the reference sequence. DNA mutation numbering was based on the cDNA sequence that used the A of the ATG translation initiation start site as nucleotide t1. Traditional nomenclature was also included using nucleotide position 133 as the translational start site. Mutation nomenclature followed general journal and Human Genome Variation Society guidelines. Exon 9 and its 34-bp upstream intron 8 region, which contains the polymorphic polythymidine tract and polymorphic TG dinucleotide repeats, were sequenced to determine the length of the IVS8 poly (T) and TG repeats.

Results of molecular analysis revealed that the father was compound-heterozygous for ΔF508 (c.1652_1655del 3bp) mutation at exon 10 in one allele and p.L375F (c.1257A>C) mutation at exon 8 in the other allele of the *CFTR* gene, while

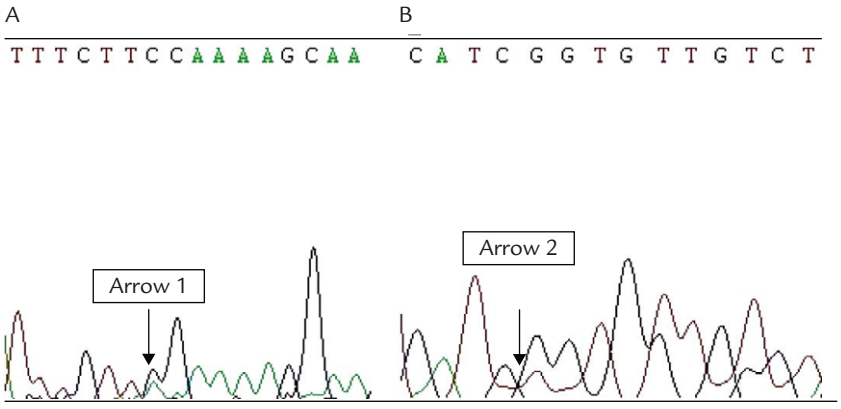

Figure 2. Sequencing results of: (A) exon 8 for son B; (B) exon 10 for son A. Arrow 1 = c.1257A > C (p.L375F). Arrow 2 = c.1652_1655del 3bp (ΔF508).

the mother was negative. The poly T of intron 8 (IVS8-Tn) of the father's *CFTR* gene was 7T/9T (Table). The TTGE mutation detection method was used to screen the two sons. The results of DNA fragments showed multiple bands on TTGE analysis for the two sons (Figure 1). Subsequent genotyping of the two sons revealed that son A was heterozygous for ΔF508, while the IVS8Tn was 7T/9T; for son B, p.L 375F and 7T/7T (Figure 2).

Discussion

Although all of the present evidence indicates that in our population, we have less risk of transmitting the mutated *CFTR* gene and a much lower risk of a child being born with CF, through interethnic marriages such as in the case reported here, the incidence of mutated *CFTR* gene carriers might increase in our population. In particular, some major mutations like ΔF508 may exist in our younger generation. Both sons in this family were found to carry the *CFTR* mutation heterozygously, because only one allele was affected. Fortunately, the mother in this study did not carry *CFTR* mutant genes (according to TTGE screening). So far, the two boys are healthy with no respiratory or digestive tract symptoms or signs. They were found to have normally developed genital organs for their age; in particular, the bilateral vas deferens was palpable and bilateral kidneys normal on renosonography. Genetic counseling was provided to the parents because the twins carry the *CFTR* gene mutation, and further follow-up will be necessary. The possibility that the *CFTR* gene mutation may be transmitted to the next generation when the children get married was also explained. The risk that CF and CBAVD in the grandchildren may occur, particularly with further interethnic marriages, was also revealed to the parents.

Counseling before ICSI for couples with CBAVD, which causes male infertility, has become essential in Western societies.[11,12] Affected triplets with one classic CF and two children with mild CF symptoms derived from a CBAVD father and a ΔF508 carrier mother has been reported.[13] Partial penetrance of the p.R117M mutation depends on the Cis-located 5T tract in intron 8 and possibly also by the nuclear-modified gene which was also found in *CFTR* mutation screening in this case. This means that the risk of CBAVD or CF transmitted by parents with CBAVD or who are carriers of the *CFTR* gene mutation may be higher than conventionally thought. In conservative Asian societies where assisted reproduction technologies such as ICSI have been developed and are becoming increasingly more frequent, genetic counseling should also be promoted to prevent hereditary hazards to the next generation through the ICSI procedure. The genetic disorder of CBAVD which causes male infertility is actually a mild form of

CF and is expressed as a variable symptom in the genitourinary tract. What is urgently needed is a program of genetic screening and counseling before ICSI because of the possibilities of affecting the next generation with the systemic lethal disease of CF.

Ethnic differences in *CFTR* gene mutations in CBAVD patients are also unique among genetic disorders causing male infertility. Compound heterozygosity for the 5T variant or mild CF mutation with a major CF mutation is considered to be a major cause of the CBAVD phenotype in Caucasians.[14] Caucasian individuals homozygous for the 7T or 9T variant will have an asymptomatic phenotype. Special characteristics of the lower frequency of *CFTR* gene mutations (36%) with none of the major *CFTR* gene mutations, such as ΔF508 or p.R117H, were found in our previous screening of Taiwanese patients for CBAVD. In the poly-T variant of intron 8, a higher frequency of the 5T allele with no 9T allele was also noted in the genetic screening of our CBAVD patients. The reasons for these genetic variations as well as the true mechanism of CBAVD and the role of the 9T variant in the pathogenesis of CBAVD remain unclear. Further exploration to identify other novel *CFTR* gene mutations or polymorphic changes in the neighboring introns in our patient population is ongoing. These new tests will also be applied to every couple with CBAVD and their siblings in a complete counseling procedure to provide more detailed information on the special genetic characteristics of *CFTR* mutations in Taiwanese.

Acknowledgments

This study was supported by a grant (SKH-FJU-94-06) from Shin Kong Wu Ho-Su Memorial Hospital, Taiwan.

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